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Using Current Data to Define New Approach in Age Related Macular Degeneration: Need to Accelerate Translational Research

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Abstract: Age related macular degeneration (AMD) is one of the major retinal degenerative disease of ageing whose complex genetic basis remains undeciphered. The involvement of various other factors like mitochondrial genes, cytoskeletal proteins and the role of epigenetics has been described in this review. Several population based AMD genetic studies have been carried out worldwide. Despite the increased publication of reports, clinical translation still eludes this davastating disease. We suggest models to address roadblocks in clinical translation hoping that these would be beneficial to drive AMD research towards innovative biomarkers and therapeutics Therefore, addressing the need large autopsy studies and combining it with efficient use of bioinformatic tools, statistical modeling and probing SNP-biomarker association are key to time bound resolution of this disease.

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INTRODUCTION

Age related macular degeneration is one of most common retinal degenerative disease among elderly individuals resulting in irreversible vision loss. Indeed, the prevalence of AMD is believed to be higher in western countries than Asian counterparts but it remains to be seen how the incidence pattern changes with fast ageing population of India, China and other countries. The pathology of AMD is distinguishable by the presence of characteristic drusen. The advanced forms of AMD have several pathological hallmarks including degeneration of retinal pigmentary cells (RPE) and formation of new vessels from underlying choroid. These outgrowths of choroidal vessels penetrate the Bruch's membrane (BM) and disrupt the integrity of RPE cell layer and their function. These pathological changes in retina impact vision and cause irreversible blindness.

Both environment and genetic factors contribute equally to the progression of AMD pathology. Several studies have defined the role of modifiable environment factors like smoking, BMI, omega-3, carotenoids, *trans*-unsaturated fat intake *etc* [1-5] which could modulate the combined effect of several genetic susceptibility factors functional in multiplicative or in additive manner [6-8]. The fundamental basis of modulation of these genetic factors may be explained by epigenetic changes in genes or on the regulatory sequences of these genes. By introducing these changes in genes or regulatory sequences it may or may not influence the functional nature of corresponding proteins. Such epigenetic changes are mostly not inheritable. On the contrary, several genetic factors alone have sufficient potential to initiate AMD pathology. Among these genetic factors, most of proteins are basically involved in metabolic processes in the cells, participate in modulating immunity of the body, especially innate immunity components, angiogenic processes, and regulatory proteins involved in apoptosis, miRNA processing (DICERs) and proteins of extracellular matrix. Gene association studies have thus been widely explored in several populations as well as by manipulating genetic makeup in animal models thus indirectly validating their association with AMD.

This review will not only shed light on different strategies underlying AMD research such as the role of mitochondrial mutations, cytoskeleton, epigenetics, lifestyle and environment that impact advancement of field but also provide insight about various roadblocks in translation of basic knowledge for clinical benefit. Why genetic association studies are not being translated for drug development shall remain the mainstay of the review. We suggest that the integrated role of bioinformatics and statistical approaches may result in unveiling the genetic complexity of AMD.

GENETICS OF AMD

Several family and twin studies have revealed the inheritability nature of and role of genetic influences on AMD pathogenesis [9-13]. The other genetic studies have shown that the first degree relatives of AMD patients have higher risk for occurrence of AMD even earlier in life as compared with first degree relatives of those without AMD [14-16]. Several population based genetic studies have demonstrated

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that several genetic susceptible loci are associated with AMD pathology. Various genetic loci which have been shown to be associated with different forms of AMD i.e. dry, wet and geographical atrophy have been listed (Table **1**). The components of innate immunity have been investigated and their role in regulation of AMD related inflammatory processes have been established. Several complement factors especially alternative complement pathway genes, including the CFH [17], component C3, Factor I, Factor B and C2, have been reported in pathogenesis of AMD. Among the dominant factors, like complement families, is the complement factor H (CFH) which regulates the alternative complement pathway. SNP analysis of CFH have shown it to be most important genetic risk factor for AMD pathogenesis. Several genetic studies on various populations have strongly shown the role of CFH in AMD pathogenesis. Most of the AMD studies related to CFH now focus on a single SNP in the coding region of CFH, Y402H –a Tyr402His substitution – as the causal variant even though the original GWAS studies analysed only the Caucasian populations. Some studies have shown that the CFH gene on chromosome 1q31 is the first major AMD susceptibility gene [18]. The association of increased risk of AMD is reported to result from the Y402H variant in exon 9 (rs1061170, T>C) [19]. The coding and non coding region of the CFH gene, which are associated with either decreased or increased risk of AMD, have also been recognized. CFH gene is known to be involved in maintaining homeostasis of complement system evident by the role of inflammation in AMD. Any disruption in this system either in the form of altered functions of CFH variants, or CFH expression is believed to induce the AMD pathology. Apart from the known Y402H variant, other CFH variants, and their haplotypes can also predispose an individual to either increased or reduced risk of AMD pathogenesis in which a major risk haplotype lie in coding region of Y402H SNP and two protective haplotypes lie in intronic variants [20]. Moreover, the association between environmental factors such as smoking and obesity, and CFH genotype have been also been examined in disease progression, including the effect of genotype on response to treatment [21]. It has even been demonstrated that monogenic inheritance of CFH variants results in deposition of drusen which could initiate RPE atrophy [22].

The 50-70% cases of AMD are influenced with complement factor genes like CFH, factor I, and C2. The SNP in these genes have been shown to be associated with progression of AMD [23]. Interestingly, very few of these studies have analysed CFH protein in serum or plasma, let alone examine SNP-protein associations or carried out SNP risk factor association analysis. Besides, majority of these studies are cross sectional, not longitudinal.

Regarding other complement factors, meta-analysis study by Thakkinstian *et al.* pooled data from 19 studies which took place between 2006 and 2011 for 4 SNPs: rs9332739 and rs547154 for C2 gene and rs4151667 and rs641153 for CFB gene, suggesting that these alleles contribute to lowering the risk of AMD pathogenesis in Caucasian population by 2.0% to 6.0% [30]. Recently, it has been reported that complement factor B polymorphism (R32Q) correlates with early AMD but have protective effect on late AMD as seen in Caucasian population [31]. On the contrary, it has been reported in INDEYE study that polymorphisms in ARMS2/HTRA1 locus are significantly associated with early and late AMD but instead of this locus the complement factor components like C2, CFH and CFB have not been found to be associated with AMD [32]. Additionally, we have reported several proteins involved in apoptosis and angiogenesis to be altered in AMD patients signifying the role of associated genetic loci and their expression in pathology. These include VEGFR2, eotaxin-2, CCR-3, DCR1, SOD1, CCL-2 *etc.* [27, 29, 33-35].

Table 1. Several non-GWAS gene loci and their association with various forms of AMD.

Dry AMD			
Gene	**Locus**	**p-value**	**Reference**
SELP	rs3917751	0.0029	24
Geographical Atropy			
Gene	**Locus**	**p-value**	**Reference**
TLR3 DICER	rs3775291 NA	P=0.005 NA	25 26
Wet AMD			
Gene	**Locus**	**p-value**	**Reference Number**
VEGFR2	rs1531289	0.047	27
All AMD Subtype			
VEGF CCR3	rs1413711 rs3091250	0.002 GT-0.001 TT-0.002	28 29

Apart from role of innate immunity in AMD pathology, genes related to metabolism, especially cholesterol metabolizing genes have also been widely explored in different populations throughout the world and found to be associated with AMD pathology. Holliday *et al.* conducted a GWAS meta-analysis of early AMD, reporting association of variants at the CFH, ARMS2 loci, and suggesting the polymorphisms of Apolipoprotein E (ApoE) to be associated with early AMD. This study suggested a weaker genetic effect on the risk of early AMD as compared to late AMD [36].

In 2010, a genome wide association study (GWAS) was conducted which reported an association of HDL with susceptibility with AMD. This study was conducted among 2157 cases and revealed strongest association between two genes namely LIPC and CETP with AMD [37]. However, in a recent case control study carried out in Chinese population having AMD (n=535) CETP was studied as one of the gene among 10 genes with different variants. No significant association of CETP was seen in AMD patients. It is possible that in case of genetic studies, different genes respond differently depending on the severity of disease [38] which may even vary between populations testifying to the role of gene-environment interactions.

AMD is a heterogeneous disease and the role of most of established genetic factors are inconclusive in AMD pathology, as suggested by analysis of SNP in different population based studies even though these are limited to Caucasian populations. Therefore, a common pathway for AMD disease pathology continues to elude us. Do these gene association approaches in AMD analysis provide sufficient ground for understanding AMD pathogenesis.

MITOCHONDRIA AND AMD

The burden of oxidative stress is a common feature of metabolically active tissues. Retina, being one of the metabolically active tissue in the body, remains susceptible for oxidative stress due to frequency of photochemical reactions. Photoreceptors contain large number of mitochondria resulting in increased reactive oxygen species (ROS). ROS can damage the integrity of macromolecules and organelles. Apart from this, increased ROS concentration can also damage the mitochondrial-DNA (mtDNA) which has been studied as major factor in pathogenesis of AMD [39, 40]. mtDNA damage has reduced capacity to cope with increased ROS burden as compared to nuclear DNA in the photoreceptor cells due to absence of repair mechanism except excision base and mismatch repair [40]. It has been shown that accumulation of mtDNA deletion and deficiency of cytochrome-c oxidase is often observed in photoreceptors, especially the fovea [41]. The number of deletions and rearrangements have been found to be higher in mtDNA of retina of AMD patients as compared to AMD control, suggesting that the impairment of mitochondrial DNA is directly related to AMD which is affected by microenvironment of retina [42]. However, these studies are again limited to Caucasian populations and such analyses need worldwide attention and validation in populous countries.

The Blue Mountain Eye study has revealed that haplotype H of mtDNA was more prevalent in European population and found to be protective for AMD [43]. Several SNPs of noncoding region of mtDNA at T16126C, T16126C + G13368A, A4917G +A73G, and T3197C+ A12308G was strongly associated with AMD. It is know that the SNP A4917G codes for NADH dehydrogenase, therefore, the changes in its SNP may hamper the function and consequently increase the burden of ROS inside retina [44, 45]. The extent to which these mechanisms disrupt retinal function has bearing on why AMD is not a developmental disorder.

EPIGENETICS AND AMD

The role of epigenetics have been widely explored in human cancer. The epigenetic changes serve to provide protection of genome by host restriction enzymes, regulation and activation of genes. These changes are known to be confined to CpG islands in most of eukaryotic cells. A series of CpG islands are present in our genome and regulatory sequences of genes. The epigenetic changes include: methylation, sumoylation, phosphorylation and acetylation. It has been established that environment factors and dietary factors introduce these epigenetic changes in the genome [46]. It has also been shown that the DNA methylation of genome was drastically decreased with age [46]. Recently, a monozygotic twins study carried out by L wei (unpublished data) have revealed that about 256 genes to be hypomethylated and 744 genes hypermethylated in AMD twins. The epigenetic changes could be inherited and introduced by several modulatory environmental factors especially smoking and deficiency of anti-oxidant in diets. Additional studies across populations are warranted that can examine the role of epigenetics in progression of AMD.

CYTOSKELETON AND AMD

The cytoskeleton plays crucial role in maintaining functional and intact retinal layers. Therefore, any changes in cytoskeleton coding genes are associated with pathological changes in these cells remains to be seen. The cytoskeleton and their associated proteins have already been implicated in development of neural retina and integrity of retinal cells layers. However, there are a few studies examining its biological effects in AMD models or SNP screening.

Apart from the well established genetic factors in AMD, the cytoskeleton and associated proteins could be involved in morphogenetic of choroidal neovascularization (CNV) in case of wet AMD through which proliferating endothelium cells of choroid remodel themselves in neovessels. It has been demonstrated that the defective cytoskeletons in cone photoreceptor results in macular degeneration [47]. Additionally, the initial inflammatory responses against accumulation of drusen, leakage of fluids after rupture of Bruch's membrane by recruiting macrophages cells, pericytes, or by activating complement cascade mechanism in sub-retinal space involve cytoskeleton remodeling. Moreover, changes in functionality of vascular endothelial growth factor (VEGF), along with transforming growth factor- β (TGF-β), and platelet-derived growth factor (PDGF) can activate the endothelial cells resulting in remodeling of the extracellular matrix (ECM) through activation of the components involved in structure and function. This, whole process can evoke the angiogenic cascade which leads to CNV formation. However, the precise mechanism behind cytoskeleton

mediated neovascularization and recruitment of cells is still not clear and requires multinational and interdisciplinary efforts.

EXTRACELLULAR MATRIX (ECM) PROTEINS AND AMD

In various human inflammatory diseases metalloproteinase regulation becomes disrupted. RPE is responsible for regulating the photoreceptors activity and extracellular matrix. The dysregulation in the dynamics between photoreceptors and choroid-RPE complex highlights the role of ECM. ECM is a key mediator in the pathogenesis of AMD. There are 28 Matrix metalloproteinases (MMP) [48] and 29 members in ADAM family out of which some encode proteolytically active proteins [49]. MMPs degrade different components of extracellular matrix and are regulated through TIMPs by inhibition of the function of these proteins. ECM proteins are chiefly involved in AMD. These include: TIMP3, COL8A1, COL10A1, ADAMTS9, HTRA1, TGFBR1, B3GALTL etc. However, practical functional application of majority of these genes is still not confirmed. A model depicting dynamics between ADAMTS, B3GALTL and AMD has been depicted in (Fig. **1**). ADAMTS which is on chromosome 3 has a key role in angiogenesis and cartilage degeneration [50]. Previously, it was demonstrated that ADAMTS9 is antiangiogenic metalloproteinases which is expressed in microvascular endothelial cells and inhibition of this gene results in angiogenesis and apoptosis [51]. ADAMTS9 regulates the Akt/mTOR pathway which promotes expression of genes related to glycolysis and glucose uptake by increasing the transcription factor HIF1a. The serine-threonine protein kinase Akt is a common mediator of cellular survival signals and failure of Akt-mediated signaling can cause apoptosis which can lead to photoreceptor degeneration, bruch membrane thickening, extracellular deposits, decreased permeability leading to RPE damage, causing AMD. Jomarv *et al*. demonstrated that in rd mouse, inactivation of Akt survival pathway results in photoreceptor cell death [52]. Akt signaling targets mTOR which promotes angiogenesis. Zhaw *et al*. showed that mTOR-mediated dedifferentiation of the RPE indicates photoreceptor degeneration in mice [53]. B3GALTL gene, which is also involved in glycosylation, contributes in the elongation of O-fucosylglycan on thrombospondin (TSP) type repeat (TSP are secreted ECM glycoproteins involved in the ECM and cellular interactions which is antiangiogenic). TSP-1 and TSP-2 are the known initial protein inhibitors of angiogenesis [54]. TSP-1 has been reported to be secreted by RPE which controls angiogenesis in the eye. Therefore, any impaired addition of sugar molecules to protein can disrupt the function of several proteins, which may lead to the abnormal angiogenesis resulting in AMD.

SNPs ANALYSIS AND SLOW PACE OF DRUG DEVELOPMENT IN AMD

Several genome wide association studies have been carried out to dissect the role of genes and their association with AMD pathogenesis (Table **2**). Even though the population based genetic studies in AMD are increasing every year, however, most of these SNPs have not been translated in development of human diagnostic, prognostic and pharmaceutical applications. The outcome of gene association studies appears over-rated in small sample size when compared to studies

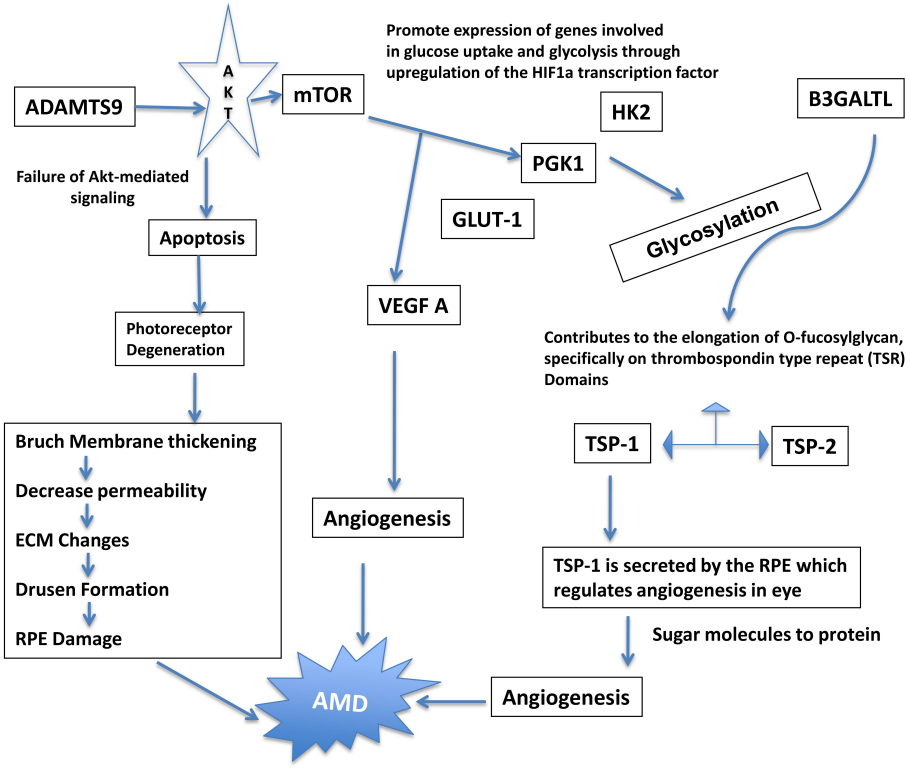

Fig. (1). Schematic representation of AMD pathogenesis mediated by the proteins responsible for integrity of extra-cellular matrix that required for function of RPE cells and imbalance in function of these proteins leads to pathology.

Table 2. Important GWAS studies throughout the world in field of AMD and their significance.

Studied Gene	Study Type	No. of Patients		p-value	Locus	Geographical Attribution	Reference Number
CFH *ARMS2* *RDBP*/*CFB*/*C2* *DNAJC27* OTOS *FSTL5*	Case Control Study	AMD Case 1207	Control 686	7.51×10^{-30} 1.94×10^{-23} 4.37×10^{-10} 6.58×10^{-6} 6.81×10^{-6} 2.70×10^{-6}	rs1831282 rs10490924 rs522162 rs713586 rs4854022 rs10857341	USA	58
CFI, C3, C9	Case- control study	1,676	745	2×10^{-8} 3.5×10^{-5} 2.4×10^{-5}	rs4698775 rs147859257 rs34882957	USA	59
Seven new loci *COL8A1-FILIP1L* *IER3-DDR1* *SLC16A8* *TGFBR1* *RAD51B* *ADAMTS9* *B3GALTL*	Case control	>17,100	>60,000	$4 X 10^{-13}$ $2X10^{-11}$ $2X10^{-11}$ $3X10^{-11}$ $9X10^{-11}$ $5X10^{-9}$ $2X10^{-8}$	rs13081855 rs3130783 rs8135665 rs334353 rs8017304 rs6795735 rs9542236	USA	60
CFH ARMS2 ApoE GLI3 GLI2 TYR	Case Control Study	4,089	20,453	1.5×10^{-31} 4.3×10^{-24} 1.1×10^{-6} 8.9×10^{-6} 6.5×10^{-6} 3.5×10^{-6}	rs2075650 rs2049622 rs6721654 rs621313	Australia	61
ARMS2/ HTRA1	Case Control Study	2594	4134	4.3×10^{-9} 7.4×10^{-14}	rs10490924	USA	62
ARMS2-HTRA1 CFH C2-CFB C3 CFI VEGFA LIPC	Case Control Study	893	2199	2.7×10^{-72} 2.3×10^{-47} 5.2×10^{-9} 2.2×10^{-3} 3.6×10^{-3} 1.2×10^{-3} 0.04	rs10490924/ rs2284665 rs10801555 rs541862 rs7690921 rs7690921 rs943080 rs10468017	UK	63
CFH ARMS2 TNFRSF10A-LOC389641 REST-C4orf14-POLR2B-IGFBP7	Case Control Study	1,536	18,894	4.23×10^{-15} 8.67×10^{-29} 1.03×10^{-12} 2.34×10^{-8}	rs800292 rs3750847 rs13278062 rs1713985	Japan	64
FRK/COL10A1 VEGFA ARMS2/HTRA1 CFH CFB C3 C2 CFI LIPC TIMP3 CETP	Case Control Study	2594 (replication-5640)	4134 (replication-52174)	1.1×10^{-8} 8.7×10^{-9} 1.2×10^{-144} 5.6×10^{-138} 2.1×10^{-134} 2.9×10^{-22} 1.4×10^{-18} 4.3×10^{-12} 2.4×10^{-11} 4.6×10^{-5} 3.7×10^{-4} 1.2×10^{-4}	rs1999930 rs4711751 rs10490924 rs1061170/ rs1410996 rs641153 rs2230199 rs9332739 rs10033900 rs10468017 rs9621532 rs3764261	USA	65

(Table 2) contd….

Studied Gene	Study Type	No. of Patients		p-value	Locus	Geographical Attribution	Reference Number
RIMS3	Case Control Study	444	300	0.23	rs11208590	USA	66
ABCA4				0.66	rs2297634		
CFHR4				0.2	rs1853883		
YOD1				0.91	rs2054780		
OBSCN				0.29	rs287614		
OBSCN				0.2	rs1150910		
ROBO1				0.51	rs2055451		
SKIV2L				0.38	rs1447338		
ADCYAP1R1				0.07	rs13129209		
ARMS2				0.48	rs429608		
TEX9				0.96	rs2267742		
CTRB1				0.45	rs10275700		
METT10D				0.25	rs10274362		
DCC				0.53	rs988426		
DCC				0.17	rs2014307		
DCC				0.04	rs2713935		
FBXO15				0.58	rs6493856		
C3				0.45	rs8056814		
SEZ6L				0.65	rs4268798		
GRID2				0.37	rs9950970		
OR1Q1				0.3	rs1367634		
				0.7	rs12954274		
				0.84	rs8086078		
				0.64	rs2230199		
				0.43	rs6137194		
				0.41	rs9608466		
					rs6532378		
					rs972925		
					rs2838378		

analysing the same polymorphism in a high number sample size. The primary outcome of biased false-positive results from scientific reports can hamper or mislead the advancement of scientific knowledge and decision making in clinical and drug development settings. Despite rapid increase in SNP association studies in AMD (regardless of claims that this will stop, personal communication: Anand Swaroop), there seems to be no hope emerging from such investigations. Therefore, there is need to define the criteria for evaluation of the significance of these results. It has been observed that majority of high impact journals and editors only accept the manuscripts which present positive results. It is necessary that reviewers and editor of journals critically evaluate results, parameters involved in these studies, application of statistical expertise without remaining influenced by positive results of the reports [55-57]. In order to provide high quality of results in basic investigations of AMD, especially with regards to genetic association studies we need to address the following issues in clinical translation:

i) Scientific knowledge gap between clinicians and basic research scientist.

ii) Absence of longitudinal studies in AMD.

iii) Lack of autopsy studies to assess the gene-pathophysiology correlation.

iv) Lack of Meta-analysis in preclinical studies in various Asian, western counterparts.

v) Studies to assess both biomarker level along with their SNP analysis.

vi) Selection of statistical tools to analyse the results of these population based studies by statisticians.

vii) Uniformity in GLP practices in results analysis, selection of standard operating protocol (SOP), and randomization in experiments.

Knowledge Gap Between Practising Clinicians and Basic Scientists

This is very unfortunate drawback in education system of both developing and developed countries that the fundamental knowledge in basic research is virtually deficient among practising clinicians and the basic knowledge of human anatomy and physiology is virtually lacking among basic scientists. In order to bridge this chasm between clinicians and basic scientists, in 2003, an effort was made by National Institutes of Health (NIH) who developed a framework of priorities to optimize and equalize entire medical research portfolio [67]. It is important to implement the guidelines, training in research and analysis, revamp of education system by inclusion of both MD-PhD and PhD-MD education

systems, cultivating additional medical research scientists in both pre-clinical and clinical settings [68, 69] in order to streamline the translational outcome i.e. development of diagnostic and prognostic markers as well as therapeutic drugs [70-72]. Moreover, it is also more important that, apart from implementing research education and training programs for clinicians, it is necessary to share clinical data and findings of AMD patients with research scientists in a real time manner. This will help to evolve an understanding of AMD pathology in a precise manner and also facilitate basic and translational research. This tie up between basic research scientists and clinicians has several benefits: development of new drugs and improving the understanding of the scientific basis of AMD pathology, developing information system driven from patients care and the results to become more reliable for translation.

Association Studies Between SNP and Biomarkers

In most of the gene association studies in AMD, it has been observed that the investigators have examined either SNPs or level of a particular protein in serum or plasma. The results obtained from such reports provide only a partial understanding of the genetics of AMD. By examining both SNP as well as protein levels in serum or plasma it is possible to examine whether SNPs changes are associated with expression level of corresponding protein. Such mendelian randomization approach (an approach to rule out the biased interpretation of confounders in genetic variation which seems to have role in disease pathology) analysis can give an insight into the nature of SNP in the population and facilitate discovery of the diagnostic biomarkers for that population making the data more creditable and reliable for drug discovery programs. Mendelian randomization approach is employed in population and epidemiological studies to examine the correlation between the genetic variation and environmental factors. By this approach biased interpretation can be avoided and confounder factors used in such genetic research may increase the robustness of the data [73].

Absence of Longitudinal Studies in AMD

Most of data from AMD research has been reported from single time point analysis. The results obtained from such investigation have several drawbacks in drawing conclusions. First, it is difficult to estimate the effect of ongoing interventions on disease progression, second; protein levels may change at different time points and, finally: several experimental errors may occur in one time analysis. Therefore, the association of these protein levels with progression of disease can enable in deciphering the causative and/or prognostic potential of these biomarkers. The longitudinal follow up of these patients at a defined time interval, will enable in determining the causative effect of protein with progression of disease accurately. These results, with longitudinal follow up can facilitate the quality of research outcome from such association studies making them more reliable for clinical translational.

Meta-analysis in Preclinical Studies in Various Populations

Preclinical studies define the safety and efficacy of administering drugs before applying them on patients. Therefore, preclinical trials have their own importance in drug discovery program. The preclinical data should not be biased and influenced with false-positive results since these studies act as raw material for development of diagnostics and prognostic markers, besides providing the substrate for validation in drug pharmacokinetics. Preclinical trials of bevacizumab, ranibizumab and pegaptanib on cynomolgus monkey, rabbits, and rats have been carried out in order to evaluate the pharmacokinetics, serum bioavailability and dose toxicity after single drug is known to vary from 2.88 days to 3 days for ranibizumab, and bioavailability of these all drugs, at higher concentration, are confined in to vitreous fluid [74, 75]. Toxicity was not observed at even higher concentration of all three drugs when tested in NMDA induced retinal degeneration model of rat [76]. This data will provide the basic information about amount of dose, based on body mass index of the patient, and the suitability of route of drug administration. Therefore, meta-analysis of preclinical studies among animals, which are not even phylogenetically related to each other would be beneficial to evaluate significance of the drug effectiveness as well as the reliability of drug to avoid false-positive outcome.

Use of Statistical Tools in Genetic Association Studies

The implication of appropriate statistical tools and analysis of obtained results is one of the important points in experimental setup (both pre clinical and clinical studies). Most of degenerative diseases have equal role of confounders along with genetic factors in disease progression. Unfortunately, most of these studies do not include the significance of these confounders in disease pathology in association with genetic data. At the preclinical setup, the statistical methods should be incorporated with the active participation of collaborator in the field. Therefore, multivariate analysis of disease pathology with genetic as well as other environmental factors should be defined in the experimental procedure. Moreover, in case of animal studies, proper experimental setup, defined groups, mortality rate, other side effects of the drugs should be spelled out properly in order to reduce the biased interpretation of results.

Autopsy Studies in AMD

Another important reason argued as a cause of failed trials in AMD is the lack of autopsy studies, because it is not possible to look in to the precise mechanism in AMD pathogenesis because most of animal models are not able to recapitulate all cardinal features of AMD, besides, these models are not phylogenetically similar to humans especially in lacking macula. Additionally, the cell culture based studies are not able to reproduce the exact mechanism or the role of these genes in AMD pathology due to improper physiological complexity of tissue in relation to three dimensional human eye. Therefore, it is very challenging to examine the precise mechanism even though this is an accepted model of analysis. Hence, it is very important to expand study design by dissecting the actual role and localization of these genes in AMD pathogenesis with the hope that these studies will provide the new vistas for the discovery of diagnostic and prognostic biomarkers.

Lack of Uniformity in GLP Practice with Respect to Result Analysis, Selection of Standard Operating Protocol (SOP), and Randomization in Experiments

To avoid false-positive and biased reporting of experiments, preclinical and clinical data, it is desirable to perform all experiments under good lab practice (GLP) regulations especially when bench workers come from varying nationalities and working environments. The GLP compliance, including the stringent documentation requirements for personnel training, periodic study monitoring and quality assurance of data and resources is the hallmark of universal acceptance of data critical for translation [77, 78]. The data from various scientific reports is expected to have variations in results due to use of different methods and procedures of experiments and different techniques to conduct a given experiment. Even the tools used in data analysis of these gene association studies vary between various research groups. The variation of results may be due to wrong interpretation of results or by applying inappropriate analytical tools. Hence, it is very important to devote quality time to analyse data from research in an appropriate manner which can be applied to build basic knowledge in a particular field. It is for this reason that compliance to GLP can ensure the research results to become more valid and reproducible. These can be used as supportive studies in translational applications.

USE OF GENETIC INFORMATION IN CLINICAL TRIALS FOR AMD

Numerous clinical trials have been conducted all over the world to facilitate drug developments and clinical research. Meanwhile, DNA information is routinely collected during the trials under informed consent. However, there is a considerable gap between the genetic studies and clinical trials. The data needs to be synthesized together to better understand disease biology. For example, the National Eye Institute (NEI), of the U.S. National Institutes of Health (NIH) launched a multi-center and randomized clinical trial named the Age-Related Eye Disease Study (AREDS) in 2001 [79, 80]. The trial is designed to study if nutrients (Antioxidant Vitamins and Zinc) can help AMD patients. Over 3,600 people with different stages of AMD participated in this trial with a median follow-up period 5 years. The research group showed that high levels of antioxidants and zinc could reduce the risk of advanced AMD and its progression. Later in 2006, NEI launched another trial (AREDS2) to study if a modified nutrient formulation can reduce the risk of AMD and its progression in a cohort of over 4,000 participants [81]. In addition to the clinical and epidemiological studies, most participants in AREDS1 and AREDS2 have consented for genetic research and the DNA was collected and stored. The samples have been genotyped using commercial whole-genome genotyping platforms for multiple purposes. Merging the genetic data with the comprehensive clinical phenotypes can help researchers reach the following goals:

1) Identify genes that affect AMD progression. Clinical trials typically follow patients for a long period with multiple visits. The collected longitudinal phenotypes (e.g. AMD scale of severity) provide the necessary information to identify genes that can increase/reduce the time to advanced AMD if the genome-wide genetic data is available. The type of survival analysis can be performed to include censoring data points, which is treated as missing data in a typical case-control study. A genome-wide scan on progression time will further our understanding of AMD on top of the great success of GWAS from previous case-control studies.

2) Improve statistical models for AMD risk and progression. After we obtain a list of variants associated with AMD, we can build statistical methods for predicting the risk of AMD based on top variants together with other demographic and environmental variables. Previous literature has shown that the risk and progression of AMD can be accurately predicted [82-84]. We can further improve the prediction accuracy based on a more completed list of variants that are discovered in 1. Furthermore, we can model two eyes separately with the consideration of within-person correlation and predict the eye-level risk using clinical information from each eye.

3) Identify genes that affect individual response to treatment. In pharmacogenomics, individual response to drugs/treatment can be affected by genetic differences through biological pathways. A second generation of genome-wide scan on drug responses or pharmacological treatments will facilitate phenotypic screening in addition to drug discovery based on targeted pathways.

4) Identify the disease subgroups that can benefit from certain drugs/treatment. The effect of drugs/treatments may vary from person to person. Individual genetic profiles provide potential for researchers to identify a subgroup of patients that have better drug responses or less side effects than general populations. Therefore, we can improve the effectiveness of certain drugs/treatment.

Moreover, given the successes of genetic studies in AMD, we can apply findings to directly facilitate the design of clinical trials of AMD. For example, Hu *et al.* proposed a statistical method to reduce the cost of prevention trials by merging genetic risk scores calculated from GWAS findings with demographic factors [85]. The rationale is to enroll a subgroup of individuals with increased incidence of AMD for early stage screening, thus saving cost for patient follow-up. Their results show that using both genetic and clinical factors can reduce trial cost by 33%.

In summary, combining genetic information with longitudinal phenotypic data from clinical trials for AMD will greatly enhance our knowledge about etiology and pathology of AMD, improve existing statistical models for predicting AMD progression, provide clues for drug developments towards personalized medicine, and save cost for future clinical trials. We anticipate that the integrative analysis of genetics, genomics, and environmental factors will have a great impact on clinical practice.

BIOINFORMATICS APPROACH

The population based gene association studies have greater significance in drug discovery programs based on outcome of SNP analysis in a population. Bio-informatics approach plays an imperative role in SNP based drug discovery settings. Several bioinformatic approaches are being adopted to signify and confirm the nature of SNPs of these

synonymous and nonsynonymous SNP (nsSNP) changes in the genome. The results of bioinformatic analysis will provide a biological annotation of nsSNP in the candidate genes thus enabling prediction of the impact of variation in structure and function of proteins. Disease risk can be predicted based on effect of nsSNPs and by analyzing its role in functional protein in the early age of the AMD patients who will be likely to develop AMD in the later stage of their life.

The amino acid sequence of a protein provides valuable information with regard to its biochemical features. The primary structure of the proteins holds the key for its higher order of conformation which will ultimately bring about its function. A number of similar sub-structures, known as domains, occur in many functionally related or unrelated proteins. Super secondary structures, also known as motifs or folds, are mostly stable arrangements of numerous elements of secondary structure. The particular order of amino acid sequence of a protein can provide the knowledge of 3-dimensional structure and function of the protein. The nature of protein, whether it is secretary, membranous or targeted for various intracellular organelles, depends on their tagged signal sequences. Mainly, these signal sequences are present on N-terminal of the peptides. Several post-translational modifications also occur in these proteins by addition and removal of several functional groups such as phosphate, acetate, various lipids and carbohydrates. It is important to map nsSNPs onto these features of proteins.

Further, It may be interesting to study the conservative/non-conservative nsSNP across classes of amino acids (I- R, K, H; II- D, E; III- A, G, I, L, M, N, P, Q, S, T, V; IV- Y, F, W) and changes from any amino acid residues (R, K, H, D, E, A, G, I, L, M, N, P, Q, S, T, V, Y, F and W) to C and vice versa. Importantly, analysis of these nsSNPs will have significant impact predicting the changes in secondary and tertiary structure of the corresponding protein using available tools. Additionally, the holistic approach to correlate these different disciplines could provide better treatment strategy to deal with such complex diseases. Recently, several studies have recommended the role of 'omics' sciences with system biology in order to provide personalized medicine to combat complex disease phenotypes [86-88]. Bowler *et al.* have combined the 'omics' approach with system biology to discover biomarkers in emphysema. They have found lower plasma and mRNA levels in PBMCs of emphysema patients that were associated with body mass index and age by using multivariate approach and have concluded the 'omics' analysis coupled with systems biology can be useful in advancement of personalized medicine [89].

The results of systems biology and bioinformatic analysis will therefore provide a biological annotation of nsSNP in the candidate genes. Thus, it will provide an insight how the changes at genomic level will reflect at the protein level, affecting protein function and progression of AMD. The study of nsSNPs in genetically associated genes will provide better understanding of the phenotype variation in AMD patients across various continents.

CONCLUSION

AMD remains a devastating and complex genetic eye disease due to the lack of effective treatments. The current treatment strategy targets the VEGF to prevent the choroidal angiogenesis which provides the symptomatic relief from the disease only in one third of the AMD cases. Some other treatment strategies are also in used in AMD based on blocking of sphingosine-1phosphate signaling process to alleviate angiogenesis [90], a variant of which already exists in cancer treatment. The review provides a critical update with attending barriers of translation in AMD research (Fig. **2**). The review recommends that systemic and 'omics' science should be merged to improve the personalized medicine approach to accelerate translation research in the field.

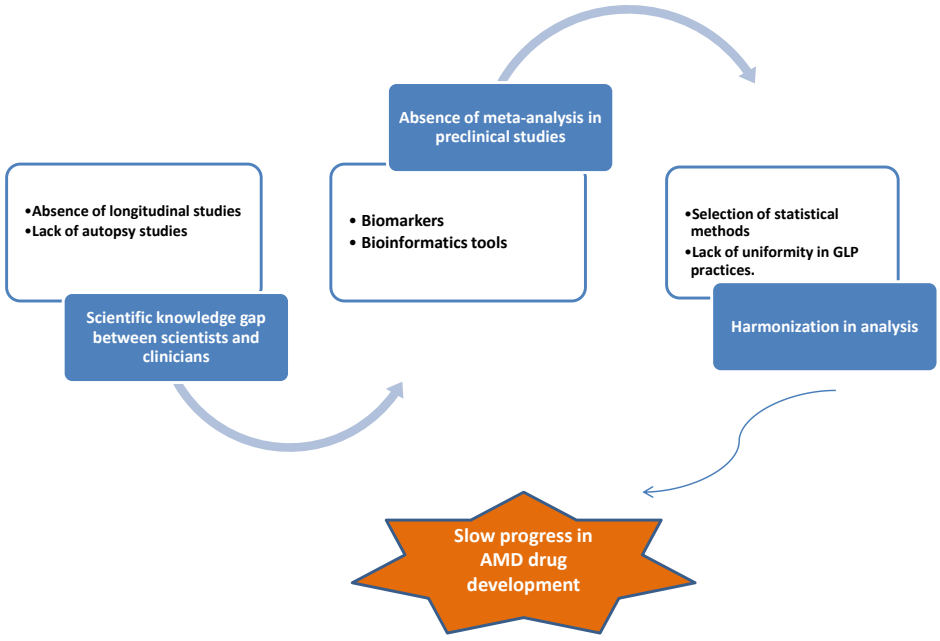

Fig. (2). Schematic representation of roadblocks in AMD translation research.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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AUTHOR'S CONTRIBUTIONS

Compiling information and initial drafting of manuscript: KS, AA, NS. Editing of manuscript: AA, WC. Concept of the manuscript: AA.

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